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Amazonian bird's nest fungi (Basidiomycota): Current knowledge and novelties on *Cyathus* species

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ABSTRACT

The genus *Cyathus* is historically subsampled in the Amazon Forest, which is a potential source of striking taxonomic richness. In this paper, a checklist of Amazonian *Cyathus* species with detailed descriptions, illustrations and comments of five uncommon species are given. A new species named *C. albinus* is proposed with morphological and molecular data, being mainly characterized by a light color hirsute exoperidium contrasting with a dark brown emplacement and basidiospores ovoid to elliptical. Three other species, *C. amazonicus*, *C. earlei* and *C. triplex*, are recorded for the first time from their localities. Also, polyphyly is detected into *striatum* infra-generic group after the addiction of tropical species, but a subclade containing Amazonian species was highly supported.

1. Introduction

There is evidences that tropical forests have great diversity and richness of fungal species (Cannon & Hawksworth, 1995; Hawksworth & Rossman, 1997), and studies focusing in diverse and combined sources of biological information revealed that integrative taxonomy is, in many cases, a powerful tool to reveal new taxa (Henkel, Smith, & Aime, 2010; Silva, Cabral, Marinho, Ishikawa, & Baseia, 2013; Cabral et al., 2014a,b), further expanding the estimates of fungal diversity in these regions.

The phyto-physiognomies that compose the mosaic of biomes forming the Amazonian domain (Coutinho, 2006) extends over nine South American countries (Brazil, Peru, Colombia, Venezuela, Equator, Bolivia, British Guiana, Suriname, and French Guiana). It forms what is known as the Panamazon (Martini, 2002), defining the Amazon not by geopolitical, but by biogeomorphological factors, indeed facilitating the elaboration and implementation of measures for the effective conservation of biodiversity (Fig. 1).

In Central Amazon, rapid habitat degradation, disorderly growth of cities and the shortage of best defined land policies combined climatic changes is leading to accelerated biodiversity loss. Because of that scientific community recognizes the urgency in learning about biodiversity in this kind of megadiverse area, before the current species become extinct due to human action (Baseia et al., 2016; Fearnside, 2006; Fearnside & Graça, 2009; IBGE, 2013; Luizão & Vasconcelos, 2002; Marques & Pinheiro, 2011; Mueller, Bills, & Foster, 2004; Nelson, Fereira, Silva, & Kawasaki, 1990; Prance, Rodrigues, & Silva, 1990; Ribeiro et al., 1999; Silva, Rylands, & Fonseca, 2005).

Cyathus Haller : Pers. is a genus of gasteroid fungi that is characterized by having small-sized (ca. 5–15 mm high) basidiomata with a cup or inverted-bell shape at maturity, with small discoid packets (peridioles) inside. Due to its similarities to bird's eggs in a nest, the genus is commonly called "bird's nest fungi" (Brodie, 1975). Although the genus is treated in a few different families depending on the authors, phylogenetic studies indicate the bird's nest fungi (including *Cyathus*, *Crucibulum* Tul. & C. Tul., *Nidula* V.S. White, *Nidularia* Fr. and *Mycocalia* J.T. Palmer) form a clade with the

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Fig. 1. Amazonian domain boundaries (Panamazon) according to Martini (2002) and collection sities.

genus *Cystoderma* Fayod, which is tightly clustered with a core Agaricaceae Chevall., including *Agaricus* L., *Lepiota* P. Browne and *Lycoperdon* Pers. (Matheny et al., 2006). There are only limited numbers of studies, but the genus is subdivided into three infrageneric groupings by molecular methods (Zhao, Jeewon, Desjardin, Soytong, & Hyde, 2007, 2008).

Cyathus species occur in many Brazilian morphoclimatic domains (Barbosa, Cruz, Calonge, & Baseia, 2014; Baseia & Milanez, 2001; Berkeley & Cooke, 1876; Coutinho, 2006; Cruz, 2013; Cruz & Baseia, 2014; Cruz, Barbosa, & Baseia, 2012a,b; Sotão et al., 2009; Trierveiler-Pereira, Gomes-Silva, & Baseia, 2009), and it is the second most represented gasteroid genus in Amazonian Forest, with eight reported species by far (Table 1). On the other hand, the Caatingas domain, a semi-arid portion of Brazil with much greater hydric stress in comparison to the Amazonian domain, has twice the number of reported species, according to the first and the only study in this area (Cruz, 2013). Arguably, it potentially means that taxonomic richness of the genus in the Amazonian domain remains quite underestimated, since high humidity is one of the main factors for the occurrence of fruit bodies of these gasteroid fungi (Brodie, 1975). The main reason for this discrepancy may be the scarcity of specialists and funding at the Amazon Forest.

In order to assess the still neglected taxonomic richness in the Amazonian domain, this work aimed to increase knowledge about the genus *Cyathus* in the Brazilian portion of this domain by describing a new species and providing new distribution data of known species.

2. Materials and methods

Specimens were collected during field expeditions in forested areas of Central Amazon at Manaus and Manacapuru cities,

Table 1
Cyathus species referred to the Amazon Forest and their geopolitical distribution within the morphoclimatic domain.

Species	Distribution	Reference
Cyathus amazonicus	Brazil (RO)[c]	Trierveiler-Pereira et al., 2009
Cyathus berkeleyanus	Brazil (PA)[a]	Sotão et al., 2009
Cyathus helenae H.J. Brodie	Brazil (PA)[a]	Sotão et al., 2009
Cyathus limbatus	Brazil (AM)[a]	Berkeley & Cooke, 1876
	British Guiana	Brodie & Dennis, 1954
Cyathus morelensis C.L. Gómez & Pérez-Silva	Brazil (AM)[a]	Cruz, Lima, Braga-Neto, & Baseia, 2012b
Cyathus stercoreus (Schwein.) De Toni	Brazil (PA)[a]	Sotão et al., 2009
Cyathus striatus	Colombia[a]	Vasco-Palacios, Franco-Molano, López-Quintero, & Boekhout, 2005
	Brazil (PA)[b]	Sotão et al., 2009
Cyathus triplex	British Guiana[a]	Brodie & Dennis, 1954
	Brazil (PA)[b]	Sotão et al., 2009

[a] Refers to first record from the Amazonian domain.
[b] First record from the Brazilian Amazon.
[c] Description of new species. For the Brazilian samples: AM – Amazonas, RO – Rondônia, PA – Pará.

Amazonas state, Brazil (Fig. 1). Studied areas were georeferenced by Global Positioning System (GPS). Macro-morphological characteristics were obtained using a stereoscopic microscope (SMZ 1500, Nikon, Tokyo, Japan). Microscopic characteristics were obtained from slides with peridiole sections mounted and observed in 5% KOH (Brodie, 1975) under optical microscope (Eclipse Ni, Nikon, Tokyo, Japan). Both pieces of equipment have a camera attached (DS-Ri1, Nikon, Tokyo, Japan). Thirty randomly selected spores were measured under 400× magnification. Colors followed the color chart Kornerup and Wanscher (1978). Analyzed vouchers were deposited in the UFRN-Fungos and INPA herbaria. Artworks in this paper were done with aid of Gimp 2.8 and QGIS 2.8.2 software.

Phylogenetic analyses were conducted in order to compare morphologically similar species, as well as for the phylogenetic placement of the new taxa proposed in this study. DNA extractions were performed using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following manufacturer instructions; lysis buffer incubation was done overnight at 55 °C. Polymerase Chain Reactions were done in 25 µl microtubes using illustra™ PureTaq™ Ready-To-Go™ PCR Beads (GE Healthcare, Buckinghamshire, UK). The nuclear ribosomal ITS and nuc-LSU regions were amplified with previously described primers and protocols (Gardes & Bruns, 1993; Martín & Winka, 2000; Vilgalys & Hester, 1990). After purification of PCR fragments with ExoSAP-IT® (USB Corporation, OH, USA), the purified samples were sent for double stranded sequencing in MACROGEN (Seoul, South Korea) with the same primers.

Cyathus, Nidula and *Crucibulum* sequences from the ITS nrDNA region and from 28S (LSU) nrDNA region published by Zhao et al. (2007, 2008) and Martín, Cruz, Duenas, Baseia, and Telleria (2015) were retrieved from Genbank composing two datasets. These datasets were combined with those sequences generated in this study. Each dataset (ITS and LSU) was separately aligned with Muscle algorithm (Edgar, 2004) and then manually edited with MEGA 6 (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Maximum Parsimony (MP) and Bayesian phylogenetic analyses were conducted either with the ITS dataset only, and concatenated ITS+LSU dataset. Only those taxa having both ITS and LSU sequences were included in the concatenated dataset mounted in SeaView 4.6 (Gouy, Guindon, & Gascuel, 2010).

The software PAUP* (Swofford, 2002) was used for Maximum Parsimony analysis, in which trees were calculated by heuristic search with the Tree Bisection Reconnection (TBR) algorithm for

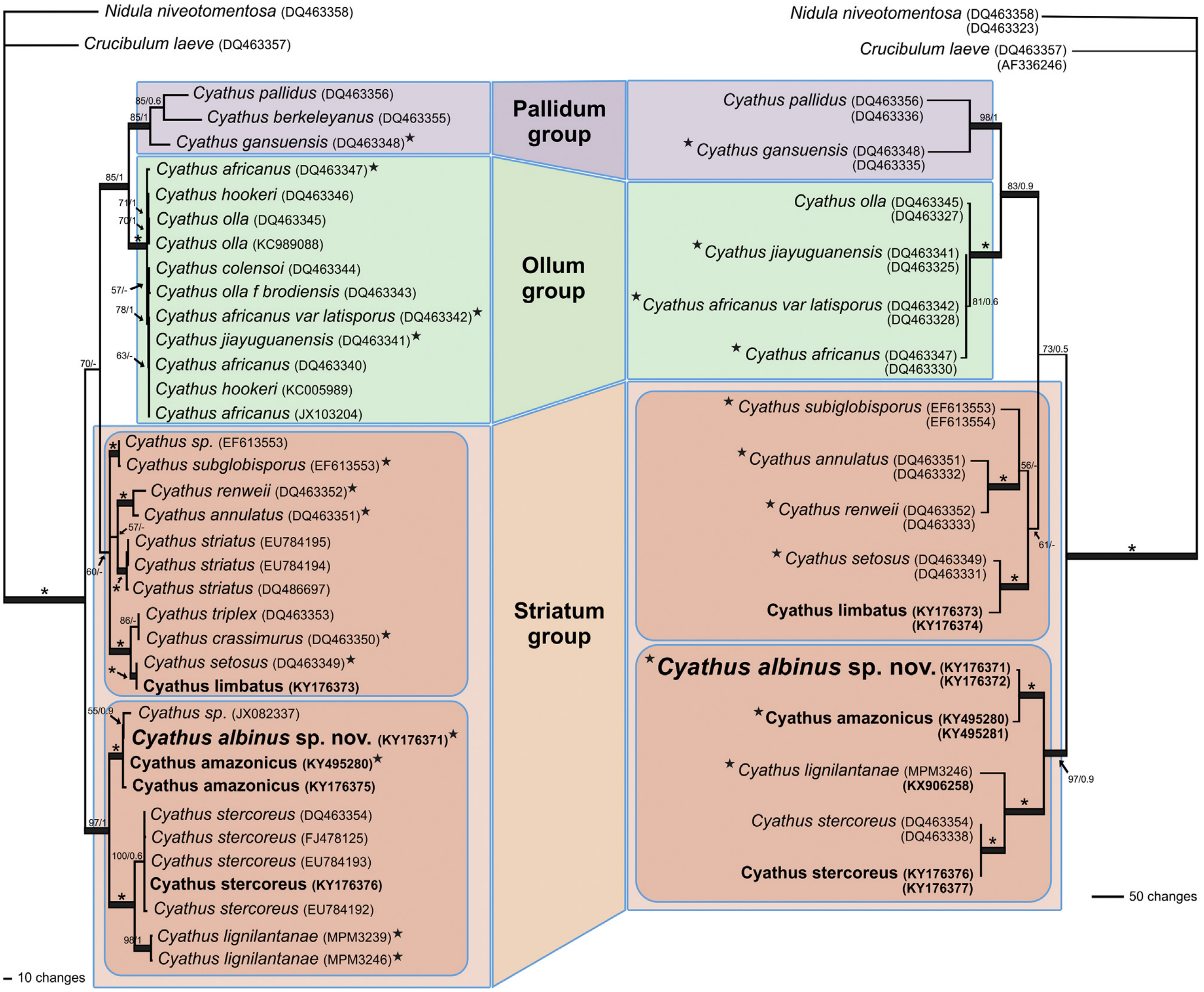

Fig. 2. Most parsimonious trees from ITS (left) and ITS-LSU (right) datasets. Numbers above branches are bootstrap support (MPbs) and posterior probabilities (PP) values. High supported branches (MPbs > 85 and PP > 0.9) are thick lined. Asterisks (*) denote branches with maximum MPbs and PP values. Taxa newly included in phylogenetic analyses by this study are in bold, sequences from type material are indicated with stars (★).

Table 2
Comparison between *Cyathus albinus* and some related species.

		C. albinus	*C. earlei*	*C. subglobisporus*	*C. pallidus*	*C. limbatus*	*C. amazonicus*
Basidiome	Size (height)	6–8.5 mm	7–9 mm	7–10 mm	5–7 mm	7–12 mm	9–11 mm
	Shape	Regularly obconical	Regularly obconical	Regularly obconical	Shortly obconical	Regularly obconical	Obconical, expanding at the mouth
Exoperidium	Colour	Light colored	Light colored	Light colored	Light colored	Brown colored	Brown colored
	Surface	Strigose tufts; plicate	Lanose tufts, plicate	Strigose tufts; plicate	Strigose tufts; smooth	Strigose tufts; plicate	Strigose tufts; plicate
Peridiole	Size and color	1.8–2.6 mm; brownish gray	2–2.5 mm; brownish gray	1.5–2 mm; greyish brown to pale brown	2 mm; dark grey to black	1.5–3 mm; brownish gray	1.7–3 mm; shiny dark gray
	Cortex	Double layered	Double layered	Double layered	Single layered	Double layered	Double layered
Basidiospore	Shape and Size	14.8–20 × 10.4–14.3 μm; ovoid to ellipsoid	15.2–19 μm × 10.2–11.4 μm; elliptic to elongated	13–18 × 12–16 μm; subglobose, rarely broadly ellipsoid	4–7.5 × 6.3–15 μm; ellipsoid to subglobose	15.1–19.3 × 10.7–16.4 μm; ovoid to subglobose	14–19 × 10.9–16.1 μm; subglobose to broadly ellipsoid
	Walls	0.8–1.3 μm thick	1.9–2.5 μm thick	1.5–2(−3) μm thick	1.2 μm thick	2.7–4.1 μm thick	2.3–3.6 μm thick

branch-swapping, collapsing branches if maximum length is zero, and *Multrees* option in effect. The initial trees were obtained by simple stepwise addition, and bootstrap of 10,000 replicates; gaps were treated as a new state. The consistency index, CI, homoplasy index, HI (Kluge & Farris, 1969), retention index, RI (Farris, 1989), and rescaled consistency index, RC (Kluge & Farris, 1969), were obtained.

Bayesian analyses were conducted with MrBayes v.3.1.2 (Huelsenbeck & Ronquist, 2001), with substitution models chosen by MrModeltest (Nylander, 2004) for each partition. This analysis consisted of three different runs with twelve simultaneous MCMC simulations over 2 million generations. Trees were sampled every 200 generations. Trees that resulted from both analyses were edited with FigTree (http://tree.bio.ed.ac.uk/software/figtree/) and CorelDraw® Graphics Suite X7. Alignment datasets and analysis data are available in TreeBase under ID 20804 (http://purl.org/phylo/treebase/phylows/study/TB2:S20804).

3. Results and discussion

3.1. Molecular and morphological analyses

The ITS dataset was composed by 38 sequences, 4 newly generated in this study and 34 obtained from GenBank. The alignment resulted in 791 unambiguously aligned nucleotide positions; 337 constant, 135 parsimony uninformative and 319 parsimony informative characters. Parsimony analysis resulted in only one most parsimonious tree (Fig. 2) with 983 steps; Consistency Index (CI) = 0.711, Retention Index (RI) = 0.861, Rescaled Consistency Index (RC) = 0.612, Homoplasy Index (HI) = 0.289).

Concatenated ITS-LSU dataset was composed by 18 taxa; 36 sequences, 8 newly generated in this study. The alignment resulted in 1582 unambiguously aligned nucleotide positions (784 from the ITS region, and 798 from the nuc-LSU region); 1025 constant, 202 parsimony-uninformative, and 355 parsimony informative characters. Parsimony analysis resulted in only one most parsimonious tree (Fig. 2) with 1020 steps (CI = 0.764, RI = 0.771, RC = 0.589, HI = 0.236).

In the Bayesian analysis, first 4800 and 6600 trees were discarded in ITS and ITS-LSU analysis, respectively, observing the average standard deviation of split frequencies dropping below 0.01; majority rule consensus tree and posterior probabilities were estimated with the 25,203 and 23,403 remaining trees in ITS and ITS-LSU analysis, respectively. Potential scale reduction factor (Gelman & Rubin, 1992) converged to 1.0 in all parameters given by MrBayes and the estimated sample size were all much higher than 100. In the ITS analysis, consensus tree of 50% majority rule averaged lnL = −5300.77, whereas in the ITS-LSU analysis, lnL = −3705.34. Tree topologies generated by Maximum Parsimony and Bayesian analyses were similar, showing equivalent clustering pattern.

The inferred tree topologies were in conflict with the previous phylogenetic studies of *Cyathus* (Martín et al., 2015; Zhao, Desjardin, Soytong, & Hyde, 2008, 2007). Most notably, the *striatum* group proposed by Zhao et al. (2007) appears polyphyletic as more taxa from the tropics were added. The result of a poorly supported second clade (MPbs = 61, PP < 0.5) in *striatum* group (Fig. 2) encompassing taxa from diverse biogeographical areas (*C. subglobisporus* R.L. Zhao, Desjardin & K.D. Hyde, *C. annulatus* H.J. Brodie, *C. renweii* T.X. Zhou & R.L. Zhao, *C. setosus* H.J. Brodie and *C. limbatus* Tul. & C. Tul.) rekindles that more studies with better geographical sampling are needed in order to understand infrageneric relationships within *Cyathus*.

The highly supported (MPbs = 97%, PP = 1 in ITS tree and; MPbs = 97%, PP = 0.9 in ITS-LSU tree) clade containing *Cyathus* *albinus* Accioly, R. Cruz & Baseia within *striatum* group (Fig. 2) is composed mainly by recently discovered tropical species which were not included in previous studies. Specifically, the subclade where *C. albinus* is inserted, along with *C. amazonicus* Trierv.-Pereira & Baseia and *Cyathus* sp., is also highly supported (maximum values of MPbs and PP in both analysis) and composed only by Amazonian taxa. An exclusively Amazonian subclade may be an announcement of great *Cyathus* diversity yet to be known, notably in neotropics.

Phylogenetic trees showed *Cyathus* sp. UFRN-Fungos 2239, *C. amazonicus*, and *Cyathus* sp. were clustered with very short branches. The short distances among species clustering appear to be common phenomena in *Cyathus* phylogenies, as indicated in the case of *ollum* group, also composed mainly by tropical species (Martín et al., 2015; Zhao et al., 2008, 2007). These short branches are still significant because these Amazonian species can be easily distinguished from each other by a plenty of morphological characters (Table 2).

Moreover, after rechecking the morphological features of the other Amazonian collections, we confirmed that the original description of *C. amazonicus* contained erroneous information. We therefore provide updated description of this species based on holotype and newly collected collections with morphological and molecular data.

3.2. Taxonomic treatment

Cyathus albinus Accioly, R. Cruz & Baseia, sp. nov. Fig. 3.
MycoBank no.: MB812874.

Etymology: *albinus*, due to the light color of the exoperidium in fresh basidiomes.

Diagnosis: *Cyathus albinus* is mainly characterized by its light color hirsute exoperidium, contrasting with the dark brown

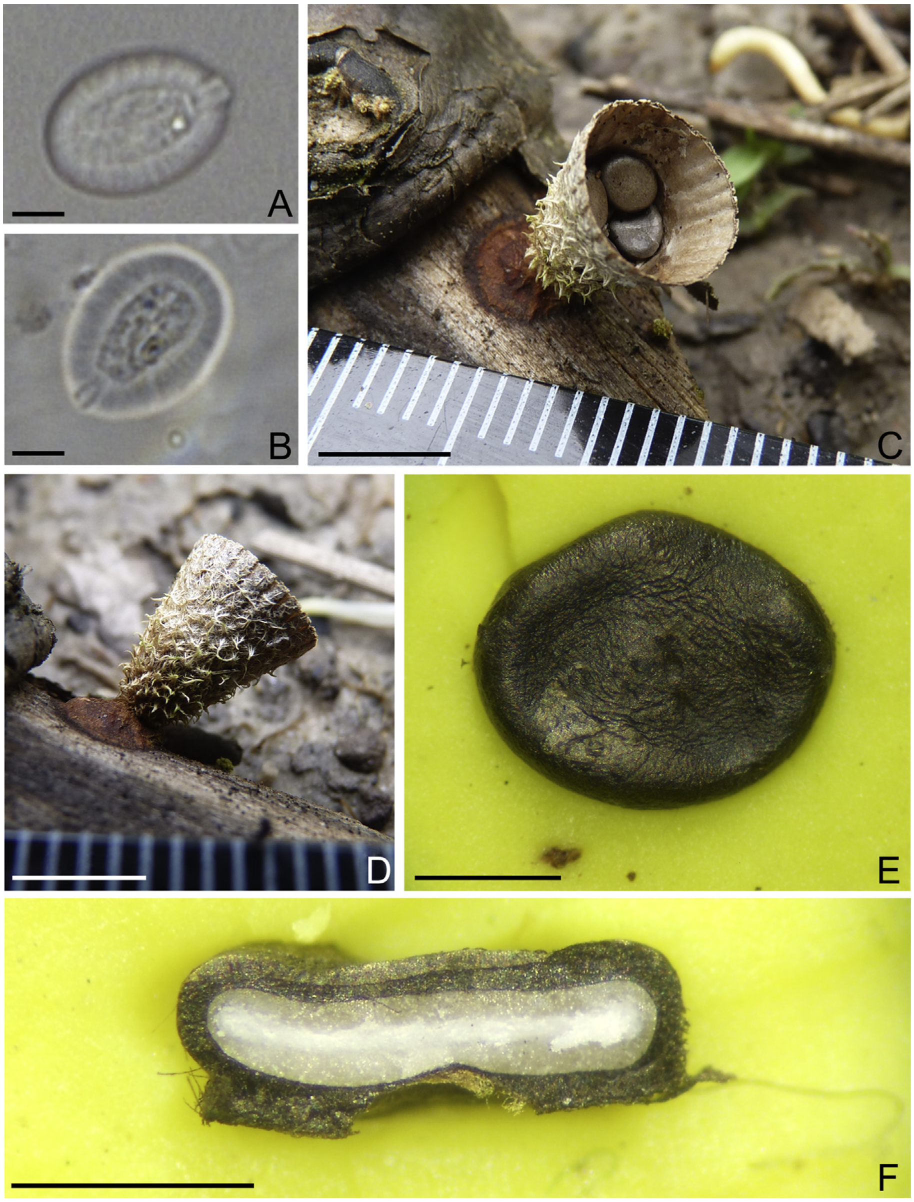

Fig. 3. *Cyathus albinus* (holotype, UFRN-Fungos 2239). A, B: Basidiospores. C, D: Fresh basidiome. E: Peridiole. F: Transversal section of peridiole showing the double-layered cortex. *Bars*: A, B 5 μm; C, D 5 mm; E, F 1 mm.

emplacement, basidiospores ovoid to ellipsoid with walls up to 1.5 μm.

Peridium infundibuliform, 6–8.5 mm (height) × 5–6.5 mm (width), not expanding at the mouth neither tapering towards the base. Emplacement 2–4 mm (diam), dark brown (eye brown) (7F6), conspicuous. Exoperidium hirsute, golden blond to dark blond (5C4 to 5D4); tomentum 0.5–0.9 mm, arranged in well defined, regular, flexible tufts; external wall conspicuously plicate, 0.3–0.5 mm between marks; mouth finely fimbriated, 0.1–0.3 mm (height), dark brown (chocolate) (6F4). Endoperidium grayish brown (6D3), inconspicuous to conspicuously plicate, 0.3–0.6 mm between marks, perceptible bright but weak, not contrasting with the exterior. Stipe 0.8 mm, grayish brown to black (6F3). Epiphragm whitish and flexible. Peridioles 2.1–2.6 × 1.8–2.2 mm, brownish gray (7F2), 15 per basidiome, angular to irregular, rarely elliptic at the edge; tunic absent, double-layered sub-homogeneous cortex. Basidiospores smooth, ovoid to ellipsoid, hyaline, apiculous conspicuous, 14.8–20 × 10.4–14.3 μm, walls 0.8–1.3 μm thick.

Gene sequences: holotype, KY176371 (ITS), KY176372 (LSU).

Habitat and distribution: on rotten wood, gregarious, exposed to the light, Brazil.

Specimens examined: BRAZIL, Amazonas, Manacapuru, margins of Manacapuru Lake (3°14′09″S 60°53′10″W), on decaying wood, 26 Feb 2013, leg T. Accioly, T.S. Cabral 107 (Holotype, UFRN-Fungos 2239!, 5 basidiomes; Isotype, INPA 276708, 4 basidiomes).

Note: The characteristics of *C. albinus* are comprehensively showed in Table 2.

Concerning the light color of the exoperidium in fresh basidiomes, *C. albinus* resembles *C. earlei* Lloyd in its finely fimbriated mouth, exoperidium hirsute and plicated, peridiole dimensions, and double-layered cortex; however, *C. earlei* diverges from *C. albinus* by presenting basidiomata expanded at the mouth, shorter exoperidial tomentum, internal wall color contrasting with the external wall, presence of a tunic covering the peridiole, more elongated basidiospores with slightly smaller dimensions and thicker walls (Brodie & Dennis, 1954; Cruz, 2013). *Cyathus subglobisporus* and *C. pallidus* Berk. & M.A. Curtis also exhibits light coloured exoperidium with strigose tufts, but *C. subglobisporus* have larger basidiomata, smaller peridioles, and smaller and more rounded basidiospores with thicker walls. *Cyathus pallidus* differs by presenting smaller basidiomata with obconical truncate shape, smooth exoperidium, smaller and darker peridioles, single layered cortex and smaller basidiospores.

Another species that shares relevant characteristics with *C. albinus* is *C. limbatus*: with regularly infundibuliform peridium, plicate and hirsute exoperidium, peridioles with double-layered cortex and without tunic, and also compatible spore dimensions and shapes. In spite of the resemblances, *C. limbatus* differs from *C. albinus* by having a woolly emplacement, distinctly fimbriate mouth, and a darker exoperidium composed of less defined tufts than those exhibited by *C. albinus*. In addition, the endoperidium color in *C. limbatus* is more brownish, the peridioles are darker and slightly smaller, and the basidiospores have thicker walls than those of *C. albinus* (Baseia & Milanez, 2001; Brodie & Dennis, 1954; Cortez, 2009; Cruz, 2013).

Cyathus amazonicus was demonstrated to be clustered together with *C. albinus* with short branches in phylogenetic trees (Fig. 2), but *C. amazonicus* differs from *C. albinus* by producing bigger basidiomata expanding at the mouth. *Cyathus amazonicus* is also characterized by having brown colored basidiomata, bigger peridioles, and more rounded basidiospores with thicker walls.

Cyathus amazonicus Trierv.-Per. & Baseia, *Mycotaxon* 110: 74 (2009). Fig. 4.

MycoBank no.: MB513133.

Peridium infundibuliform, 8–9 mm (height) × 6.5–7 mm (width), expanding at the mouth and tapering abruptly towards the base. Emplacement 2–4 mm (diam), dark brown (6F4), conspicuous. Exoperidium hirsute, grayish brown (6F3); with yellowish brown tomentum (5E4), 0.6–1 mm, arranged in irregular, rigid tufts; external wall conspicuously plicate, 0.3–0.5 mm between marks; mouth distinctly fimbriated, 0.4–0.5 mm (height), grayish brown (6F3). Endoperidium grayish brown to dark brown (7E3 to 7F6) (brownish orange when fresh; 6C3), conspicuously plicate, 0.4–0.6 mm between the marks, weak bright, not contrasting with the exterior, except when fresh. Stipe absent. Epiphragm whitish and flexible. Peridioles 2.3–2.7 × 1.8–2.4 mm, grayish brown (7F3), 13 per basidiome, circular to irregular at the edges; tunic absent, double-layered cortex. Basidiospores smooth, ellipsoid to subglobose, hyaline, a few with apiculous, 14.3–19.4 × 10.9–16.1 μm, walls 2.3–3.6 μm thick.

Gene sequences: holotype, KY495280 (ITS), KY495281 (LSU). Newly collected samples, UFRN-Fungos 2798, KY176375 (ITS).

Holotype: BRAZIL, Rondônia, 15 Feb 2008, leg. Gomes-Silva 429 (URM 80036!).

Habitat and distribution: on decaying palm-tree leave, gregarious, exposed to the light, Brazil; In Amazon Forest there are records from Brazil (Amazonas and Rondonia State).

Specimens examined: BRAZIL, Rondônia, Porto Velho, Cuniã Ecological Station, 15 Feb 2008, leg. Gomes-Silva 429 (holotype, URM 80036!). BRAZIL, Amazonas, Manacapuru, margins of Manacapuru Lake (3°14′09″S 60°53′10″W), on decaying wood, 27 Feb 2013, leg. T. Accioly and T. S. Cabral 117 (UFRN-Fungos 2798; INPA 276707); T. Accioly and T. S. Cabral., 26 Feb 2013, leg. T. Accioly and T. S. Cabral. 106 (INPA 276711).

Note: This uncommon species is mainly characterized by its pronounced bell-shaped basidiomata, expanded at the mouth and tapering at the base, peridium conspicuously plicate, peridioles up to 3 mm (diam), double-layered cortex, and basidiospores ellipsoid to subglobose with thick wall.

Morphological examination of type material of *C. amazonicus* showed that this species have peridioles with double layered cortex instead of single layered cortex, as mentioned in Trierveiler-Pereira et al. (2009). This information is critical, and this kind of mistake in a new species publication can generate serious taxonomic confusion, since the cortex layers of the peridiole are one of the fundamental characteristics to lead morphological species recognition in *Cyathus*.

All morphological characteristics of our collections match with the holotype collection, and both materials were collected from the same biogeographical areas. ITS sequence from our material also shown identity of 99% with ITS sequence from type material.

Cyathus amazonicus is easily distinguished from closely related species, *C. albinus* and *C. limbatus*. The former have lighter and regularly infundibuliform basidiomata and, the latter show a regularly infundibuliform basidiomata growing on a wooly emplacement. Both species have more rounded and thicker walled basidiospores (Table 2). This is the first record of this species from Central Amazon.

Cyathus earlei Lloyd, *Mycol. Writ.*: 26 (1906). Fig. 5.

MycoBank no.: MB237768.

Description: — Lloyd (1906: 26); Brodie and Dennis (1954: 158); Brodie (1962, pp. 1483–1484); Cruz et al. (2013: 367; 2014: 535).

Habitat and distribution: on decaying wood, gregarious, Brazil, Cuba, Puerto Rico, Mexico, Hawaii, Costa Rica; In Amazon Forest

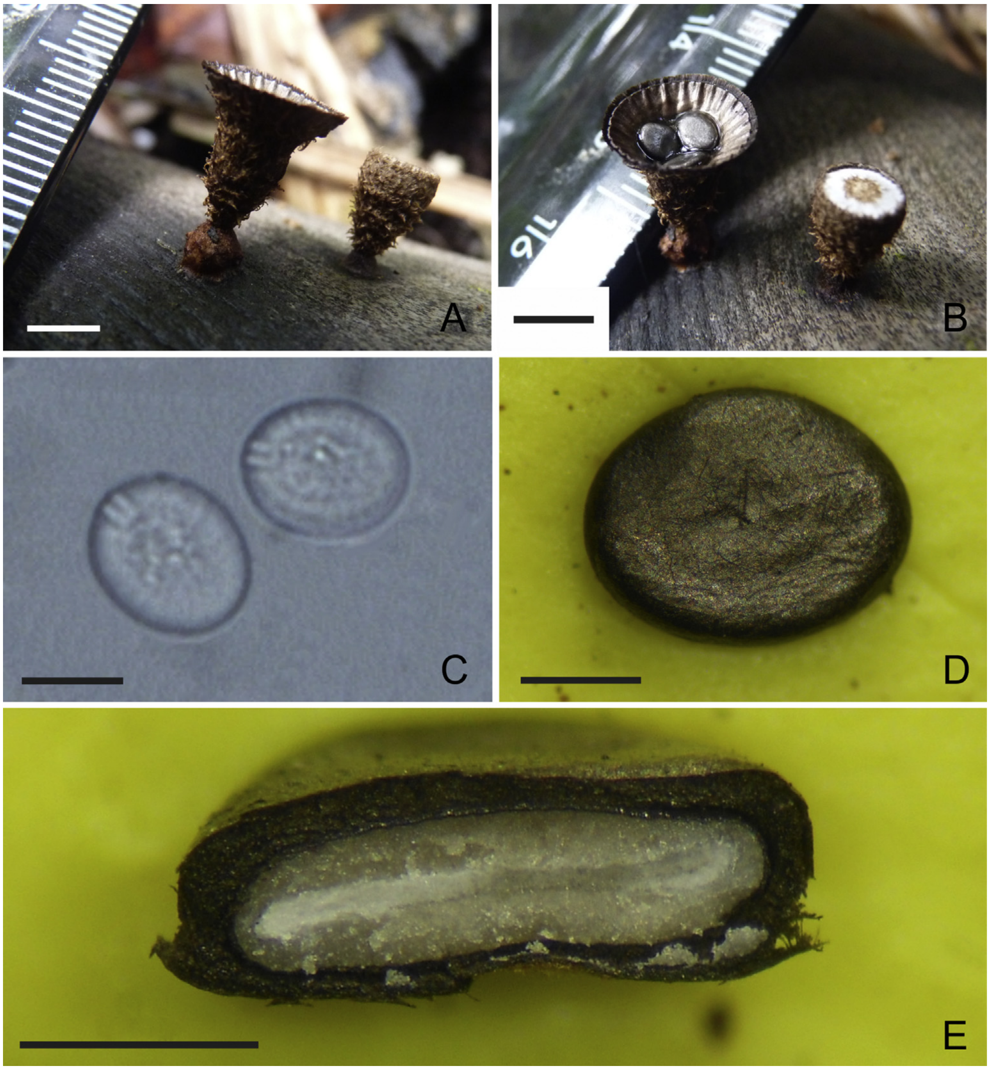

Fig. 4. *Cyathus amazonicus* (UFRN-Fungos 2798). A, B: Fresh basidiomata. C: Basidiospores. D: Peridiole. E: Transversal section of peridiole showing the double-layered cortex. *Bars*: A, B 5 mm; C 10 μm; D, E 1 mm.

there are records from Brazil (Amazonas State).

Specimens examined: BRAZIL, Amazonas, Manaus, Campus III of INPA (3º05′31″S 59º59′39″W), on decaying wood, 17 Feb 2013, leg. T. Accioly, I. G. Baseia and N. K. Ishikawa 093 (UFRN-Fungos 2237); T. Accioly, I. G. Baseia and N. K. Ishikawa., Manacapuru, margins of Manacapuru Lake (3º14′09″S 60º53′10″W), on decaying wood, 26 Feb 2013, leg. T. Accioly and T. S. Cabral 105 (INPA 276706).

Note: From *Cyathus earlei*, ITS nrDNA sequence was not obtained; this species was excluded from the final analyses. The LSU nrDNA of the sample UFRN-Fungos 2237 has been located at the GenBank with the accession number KY964272.

Cyathus earlei (Fig. 5) belongs to the *olla* group, proposed by Brodie (1975), and is characterized by an exoperidium without plication, covered by a light color tomentum, organized in small tufts, endoperidium smooth or inconspicuously plicate, with a light, shiny color, contrasting with the exterior layer, peridioles with double-layered cortex and tunic, spores 12.7–17.8 (−34) × 8.9–12 μm, ellipsoid to subglobose, with apiculus and walls 1.9–3.8 μm thick (Brodie & Dennis, 1954; Cruz, 2013).

There are four species similar to *C. earlei* in their tomentum and exoperidial color, characteristics of the mouth, and presence of tunic in the peridioles [*C. olla* (Batsch) Pers., *C. canna* Lloyd, *C. triplex* Lloyd, and *C. pallidus*]. Among these, *C. triplex* differs mainly by the smaller basidiomes (4–6 mm high), smaller peridioles (1.5–2 mm long) and more hirsute exoperidium (Brodie, 1975; Cruz, 2013). The other three species exhibited macroscopically similar exoperidium, and compatible basidiomata sizes. However, *C. pallidus* has smaller peridioles with single-layered cortex, and smaller basidiospores (5–10 × 4–6.3 μm) (Baseia & Milanez, 2001; Cruz, 2013). *Cyathus olla* exhibits basidiospore shapes similar to those of *C. earlei*, but differs by having a darker endoperidium (brownish), bigger peridioles (2–3.5 mm long) with single-layered cortex, and smaller basidiospores (6–13 × 6–8 μm) (Baseia & Milanez, 2001; Cortez, 2009). *Cyathus canna* differs by having globose basidiospores with smaller dimensions (7–9 μm) (Brodie & Dennis, 1954).

Another species that resembles *C. earlei* in the light color of the fresh exoperidium is *C. fimicola* Lloyd, but the latter exhibits smaller basidiomes (4–5 mm in height) and darker peridioles than those of *C. earlei* (Brodie & Dennis, 1954). *Cyathus earlei* has already been reported from other areas of Brazil, such as the Atlantic rainforest (Cruz et al., 2012a) and Highland forest (Cruz, 2013), but this is the first record of this species from the Amazon forest.

Cyathus limbatus Tul. & C. Tul., *Annls Sci. Nat.*, Bot., sér. 3 1: 78

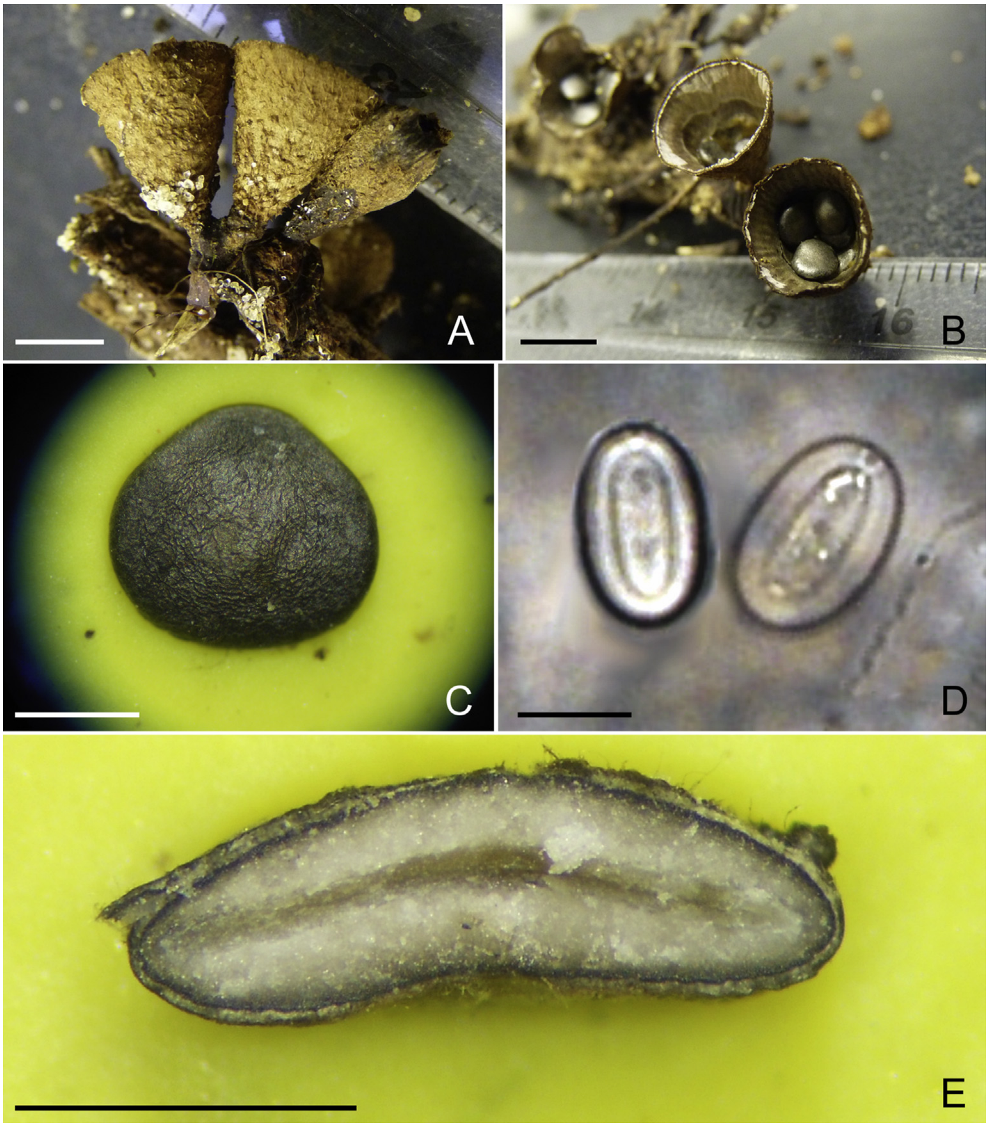

Fig. 5. *Cyathus earlei* (UFRN-Fungos 2237). A, B: Fresh basidiomes. C: Peridioles. D: Basidiospores. E: Transversal section of peridiole showing double-layered cortex. *Bars*: A, B 5 mm; C, E 1 mm; D 10 μm.

(1844). Fig. 6.

MycoBank no.: MB240889.

Description: — Brodie and Dennis (1954: 155); Cruz, Assis, and Baseia (2014: 535); Tulasne and Tulasne (1844: 79).

Gene sequences: newly collected samples, UFRN-Fungos 2238, KY176373 (ITS), KY176374 (LSU).

Habitat and distribution: on decaying wood, gregarious, Brazil, British Guiana, Suriname, India, China, Hawaii, Central and South Pacific. In Amazon Forest there are records from Brazil (Amazonas) and British Guiana.

Specimens examined: BRAZIL, Amazonas, Manaus, Adolpho Ducke Forest Reserve, Manaus Botanical Garden (3°00′14″S 59°56′22″W), on decaying wood, 19 Jan 2013, leg. T. Accioly, N. K. Ishikawa and R. Vargas-Isla 025 (UFRN-Fungos 2238); T. Accioly, N. K. Ishikawa and R. Vargas-Isla., 026 (INPA 276696); T. Accioly, N. K. Ishikawa and R. Vargas-Isla., 22 Jan 2013, T. Accioly, N. K. Ishikawa and R. Vargas-Isla. 034 (INPA 276697); T. Accioly, N. K. Ishikawa and R. Vargas-Isla., 039 (INPA 276698); T. Accioly, N. K. Ishikawa and R. Vargas-Isla., 16 Feb 2013, leg. T. Accioly, N. K. Ishikawa, I. G. Baseia and T. S. Cabral 063 (INPA 276700); T. Accioly, N. K. Ishikawa and R. Vargas-Isla., Manacapuru, margins of Manacapuru Lake (3°14′09″S 60°53′10″W), on decaying wood, 26 Feb 2013, leg. T. Accioly and T. S. Cabral 111 (INPA 276709); T. Accioly, N. K. Ishikawa and R. Vargas-Isla., 27 Feb 2013, T. Accioly, N. K. Ishikawa and R. Vargas-Isla. 121 (INPA 276712).

Note: *Cyathus limbatus* (Fig. 6) is characterized by its relatively large basidiomata (7–12 mm high) growing on a conspicuous, brown and woolly emplacement, plicate peridial layers, hirsute exoperidium formed by brown mycelial tufts (0.5–0.7 mm in length), peridioles with double-layered to subhomogeneous cortex, and ovoid to ellipsoid basidiospores (13.9–20 × 9–14 μm) with thick wall (up to 4 μm) (Baseia & Milanez, 2001; Brodie & Dennis, 1954; Cortez, 2009; Cruz, 2013).

Four species exhibit basidioma size and exoperidial characteristics similar to *Cyathus limbatus*: *C. poeppigii* Tul. & C. Tul., *C. striatus* (Huds.) Wild, *C. montagnei* Tul. & C. Tul., and *C. berkeleyanus* (Tul. & C. Tul.) Lloyd. Among them, *C. berkeleyanus* differs by having slightly smaller and darker peridioles, tunic, and single-layered cortex, in contrast to the double-layered cortex of

Fig. 6. *Cyathus limbatus* (UFRN-Fungos 2238). A–C: Fresh basidiomata. D: Peridiole. E: Basidiospores. F: Transversal section of peridiole showing subhomogeneous double-layered cortex. *Bars*: A–C 2 mm; D, F 1 mm; E 10 μm.

the *C. limbatus* peridioles (Baseia & Milanez, 2001; Brodie & Dennis, 1954; Cortez, 2009).

Cyathus poeppigii and *C. montagnei* resemble *C. limbatus* with their hirsute and plicate exoperidium. They also exhibit double-layered and subhomogeneous cortexes, respectively, although *C. montagnei* has previously been reported to exhibit peridioles with an apparently single-layered cortex (Baseia & Milanez, 2001). In spite of their similarities, both species differ from *C. limbatus* by presenting slightly larger peridioles and divergent basidiospores with subglobose forms and a larger size in *C. poeppigii* [22–34.3 × 17–22.8 μm], and also having more variable dimensions in *C. montagnei* [(15–)17–20(–25) × 8–12.5 (–20) μm]. Furthermore, *C. poeppigii* and *C. montagnei* both have finely fimbriate mouths, while *C. limbatus* exhibits more conspicuously fimbriate mouth. Additionally, *C. poeppigii* shows longer tomentum (1–1.5 mm) than *C. limbatus*, and *C. montagnei* can exhibit less

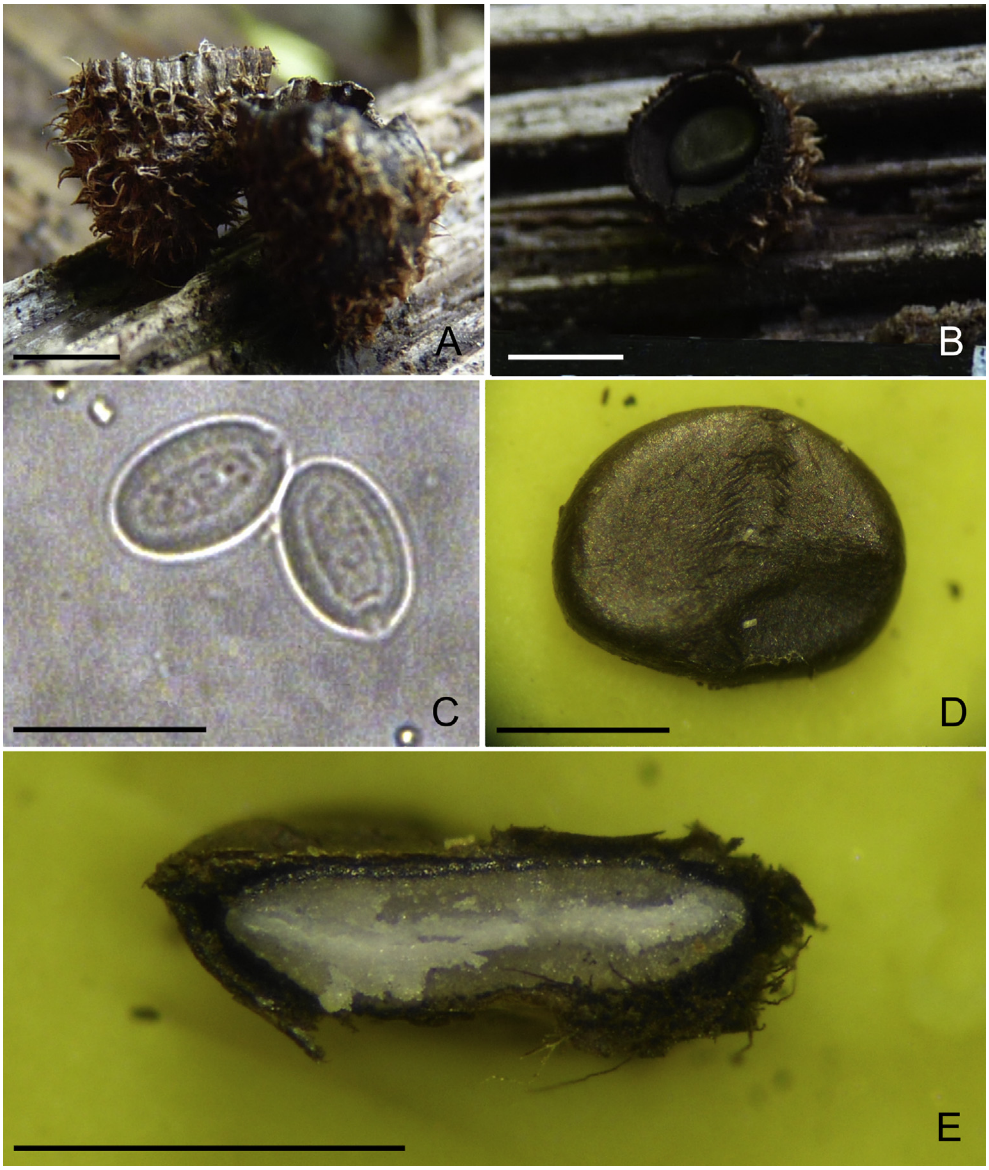

Fig. 7. *Cyathus triplex* (UFRN-Fungos 2240). A, B: Fresh basidiomata. C: Basidiospores. D: Peridiole. E: Transversal section of peridiole showing the double-layered cortex. *Bars*: A, B 2 mm; D, E 1 mm; C 20 μm.

conspicuous plication in the exoperidium (Baseia & Milanez, 2001; Brodie, 1975; Cortez, 2009; Cruz, 2013).

Cyathus striatus is also similar, but does not exhibit basidiomes with such irregularly infundibuliform shapes as those of *C. limbatus*. Moreover, basidiomata of *C. striatus* exhibits an abrupt expansion near the mouth, becoming abruptly slender toward the base, or exhibit a campanulate format. Other diverging characteristics of *C. striatus* include darker peridioles that are composed of a single-layered cortex, and the basidiospores with more elongated shapes without an apicule (Baseia & Milanez, 2001; Cruz, 2013).

Cyathus triplex Lloyd, *Mycol. Writ.*: 23 (1906). Fig. 7.
MycoBank no.: MB211281.

Description: — Lloyd (1906: 23); Brodie and Dennis (1954: 158); Zhou, Zhao, Zhao, and Chen (2004: 250).

Habitat and distribution: on rotten palm-tree leave, lignicolous, solitary to gregarious, Brazil, British Guyana, Puerto Rico, EUA, Hawaii, Venezuela, Mexico, Philippines and Thailand. In Amazon Forest there are records from Brazil (Amazonas and Pará State) and British Guiana.

Specimens examined: BRAZIL, Amazonas, Manacapuru, margins of Manacapuru Lake (3º14′09″S 60º53′10″W), 26 Feb 2013, leg. T. Accioly and T. S. Cabral 112 (UFRN-Fungos 2239); T. Accioly and T. S. Cabral., 113 (INPA 276710).

Note: *Cyathus triplex* (Fig. 7) is characterized by having reduced basidioma size (4–6 mm high), hirsute, smooth to inconspicuously plicate exoperidium, smooth endoperidium, dark peridioles with double-layered to subhomogeneous cortex and tunic present, basidiospores 12.7–22 × 10–15.2 μm, subglobose to ellipsoid, and walls 1–3.8 μm thick (Brodie, 1975; Cruz, 2013).

Cyathus triplex resembles *C. pallidus* by having smooth endoperidium and peridioles with similar color and size, and by having a tunic. However, peridioles of *C. triplex* have a double-layered cortex, but those of *C. pallidus* have a single-layered cortex. Moreover, *C. pallidus* differs from *C. triplex* by having a lighter exoperidium and smaller spores, varying between 5–8 × 4–5 µm (Baseia & Milanez, 2001).

Concerning the small-sized basidiomata and characteristics of the peridioles, *C. triplex* resembles *C. hortensis* R. Cruz & Baseia and *C. fimicola*. *Cyathus triplex* differs from both in its exoperidium color. Additionally, in the case of *C. hortensis,* the exoperidium and endoperidium are also conspicuously plicate. Furthermore, both species diverge from *C. triplex* with their spores, being slightly smaller (16 × 8 µm) in *C. fimicola*, and larger in *C. hortensis* (17.7–34.3 × 13–20.3 µm). *Cyathus hortensis* also diverges in the presence of a tunic covering its peridioles (Brodie & Dennis, 1954; Cruz & Baseia, 2014).

Cyathus poeppigii is another similar species to *C. triplex* concerning the basidiomata size, mouth characteristics, color, and cortical layers of the peridioles., but they differ on a markedly plicate peridium and longer tufts at the exoperidium in *C. poeppigii* (Baseia & Milanez, 2001; Cruz, 2013). *Cyathus intermedius* (Mont.) Tul. & C. Tul. also resembles *C. triplex* in the absence of plication in the exoperidium and endoperidium, cortical layers of the peridioles, and basidiospore size, but *C. intermedius* differs from *C. triplex* by having larger peridioles, setose mouth ornamentation, and larger basidioma (Cruz, 2013). This is the first record of *Cyathus triplex* from the Central Amazon and Amazonas State.

Disclosure

The authors declared no conflicts of interests.

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